5. INVESTIGATIONS ON THE BASIC BIOPOLYMER STRUCTURE OF THE ECTEXINE OF ALNUS GLUTINOSA (L.) GAERTN.

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Abstract

Partially degraded exines of *Alnus glutinosa* (L.) GAERTN. were investigated with the TEM method. The quasi-crystalloid biopolymer lattice was discovered in angstroem dimension and was investigated in ultrathin sections. Several kinds of the modified Markham rotation method were used to verify and investigate the symmetry of the basic polygon. Complementary elements of the methods are also introduced in this paper.

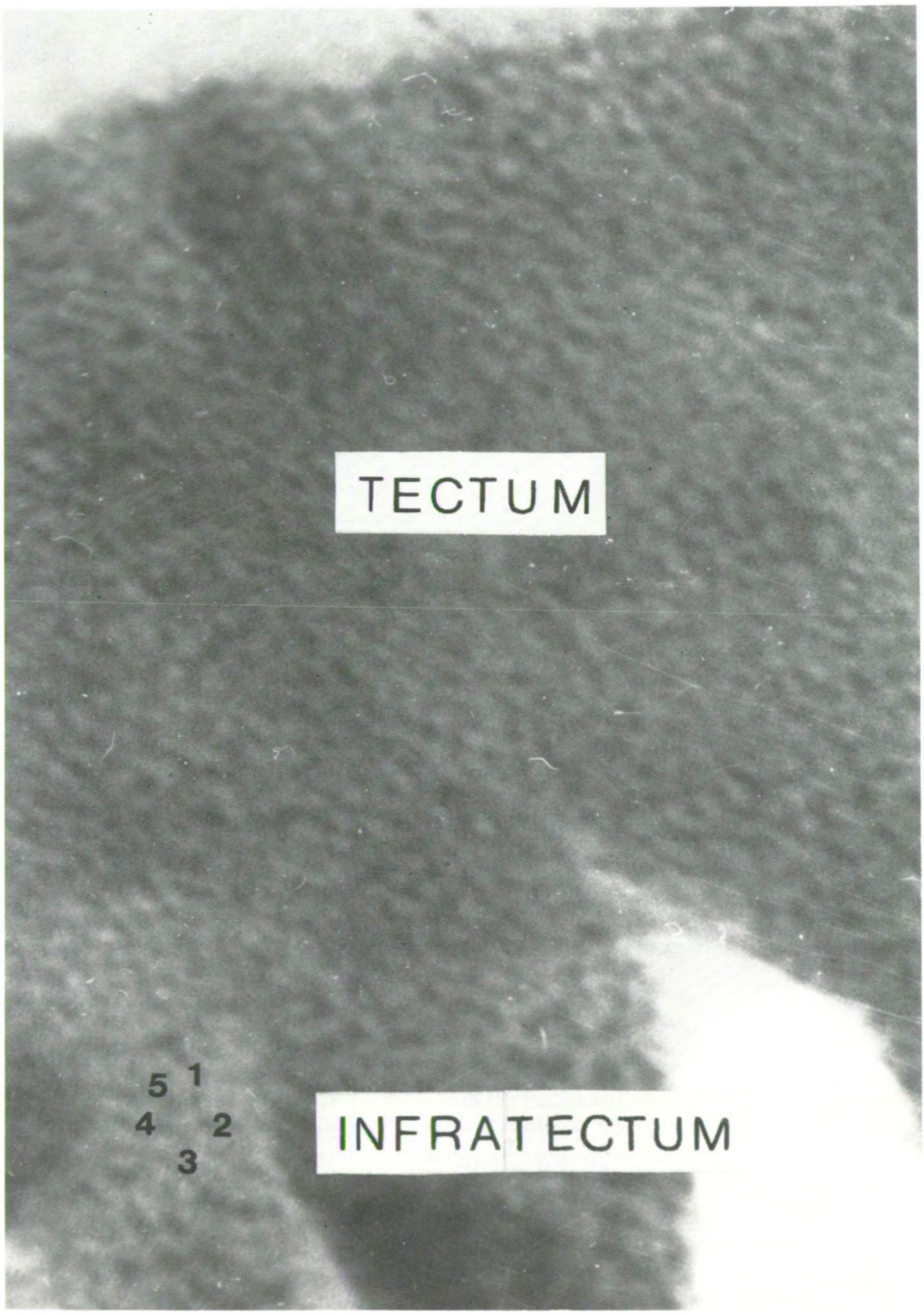
Key words: Palynology, *Angiospermae, Alnus,* biopolymer organization.

Introduction

After several data about exine sub-units published in previous publications (e. g.: ROWLEY et al., 1981, SOUTHWORTH, 1986), the quasi-crystalloid character of the basic biopolymer units of the sporoderm was published first in 1988 by KEDVES. This first observation was followed by detailed methodical and enlarged research program. We urgently needed data on angiosperm exines and other kinds of plant cell walls because the first investigations were made on gymnosperm exines. Several methodical, molecular structural and biopolymer evolutionary studies are under development. To use the fragmentation method of the partially degraded exines the pollen grains of *Alnus glutinosa* (L.) GAERTN. were chosen for the first attempt (KEDVES and ROJIK 1989).

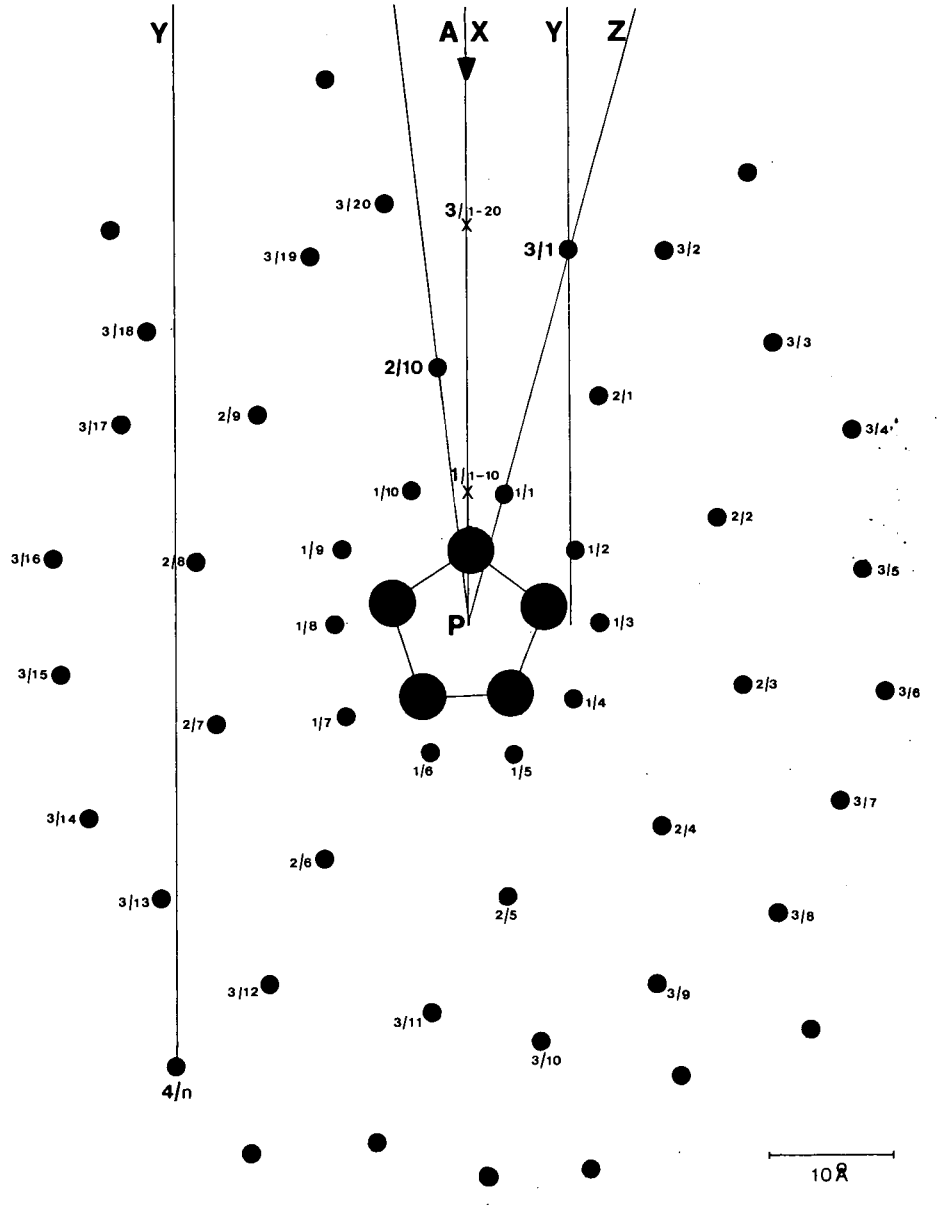
The purposes of the present investigations are the following:

1. To get information about the biopolymer organization of the partially degraded wall and angiosperm pollen grain with the transmission electron-microscope method.
2. The rotation methods were used on basic biopolymer units inside of the partially degraded wall in contrast to the first observed biopolymer unit of the exine of *Pinus griffithii* MCCLELL.
3. To compare the obtained results with the previous ones, particularly with data of the fragmentation method of this species.
4. To use critically the above mentioned methods and to modify or complete them if necessary.


TECTUM
5 1
4 2
3
INFRATECTUM

◀ Plate 5.1.

Alnus glutinosa (L.) GAERTN., partially degraded exine. The numbering of the globular elements at the edges indicates the biopolymer unit for symmetry investigations. Experiment No 226. Negative no: 8349, 500.000 x.



Text-fig. 5.1.

Schema of the biopolymer and secondary points of symmetry after rotation C.P.5.A.5.10., and the axes.

Materials and Methods

The material of investigation was collected by Dr. K. MARGÓCZY on 25th February, 1989, in the Botanical Garden of the J. A. University. The freshly collected material was frozen at 20 °C below zero. The experiment was made on 28th May in 1988 as follows. We mixed 20 mg air dried pollen grains to 1 ml 2—aminoethanol at a temperature of 30°C for 24 hours. Then we washed it with distilled water and added 10 ml $KMnO_4$ aq. dil. at a temperature of 30 °C for 24 hours length of time. After washing it, the partially degraded exines were fixed in OsO_4 aq. dil. and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made by a Porter Blum ultramicrotome with glass knives in the Electron Microscopical Laboratory of the Department of Biophysics of the Hungarian Academy of Science. The TEM pictures were made by a BS Tesla—500 transmission electron microscope in the Laboratory of Electron-microscopy of the Faculty of Sciences of the J. A. University. We express our sincere thanks to Dr. I. ROJIK for his technical assistance.

Results

TEM picture of Plate 5.1. well represents the results of the partial degradation of the pollen grain. The elements of the quasi-crystalloid lattice of the tectum (pro parte) and a part of the infratectal layer (infratectum) are illustrated. The molecular and biopolymer pecularities of the surface were not investigated during this experiment. The pentagonal polygon basic biopolymer unit was chosen at the upper part of the infratectum for symmetry investigations with the modified MARKHAM rotation method. The numbering of the globular elements at the edges indicates the AP axis, too ("A — a linear feature between the centre of the actual biopolymer and one apex of the biopolymer polygon", KEDVES 1990, p. 184). This apex is marked with number "1" every time. On that account this apex was not indicated in this picture. The scheme of the modified MARKHAM rotation operations and the secondary points of symmetries are illustrated in text-fig. 5.1.

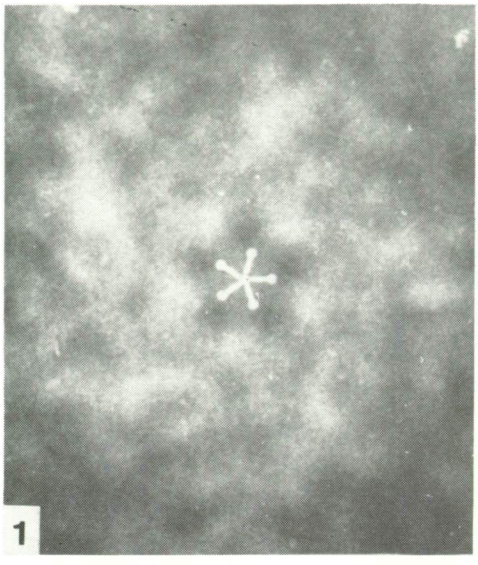
1. THE RESULTS OF THE PRIMARY ROTATIONS

C.P.5.A.5.5. (Plate 5.2., figs. 1, 3, 6, 7 and 8)

This kind of basic rotation was repeated six times for basic methodical purposes in this case. The reason of this numerous repetitions was that this basic biopolymer unit is structural and it can be found not at the border of the disintegrated biopolymer lattice as at *Pinus griffithii* MCCLELL. From these rotations we present three ones. Figs. 1, 2 in Plate 5.2. represent the best — we can say the perfect — result. This was taken as a basis for the further investigations. The shadow of the pin

Plate 5.2. ►

Alnus glutinosa (L.) GAERTN.
The basic biopolymer unit after rotation.
1. Rotation: C.P.5.A.5.5., x 1 Million.
2. Rotation: C.P.5.A.5.10., x 1 Million.
3., 6., 7., 8. Rotation: C.P.5.A.5.5., 500.000 x.
4., 5. Rotation: C.P.5.A.5.10., 500.000 x.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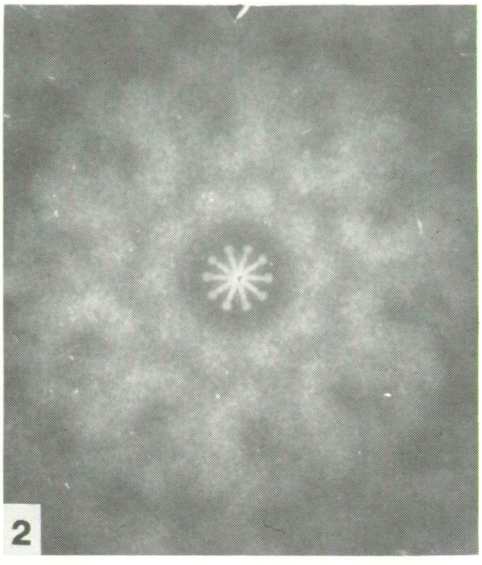
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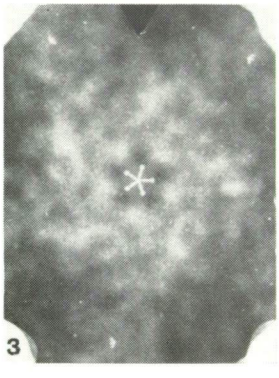
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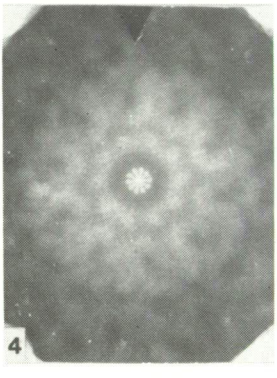
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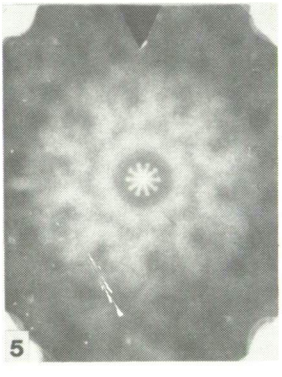
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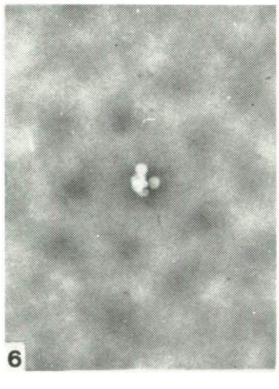
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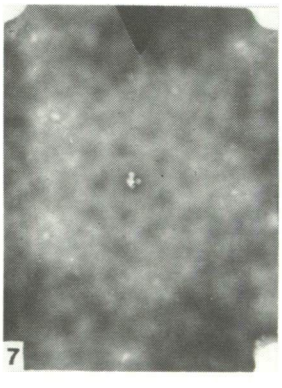
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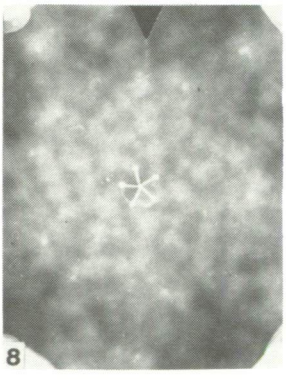
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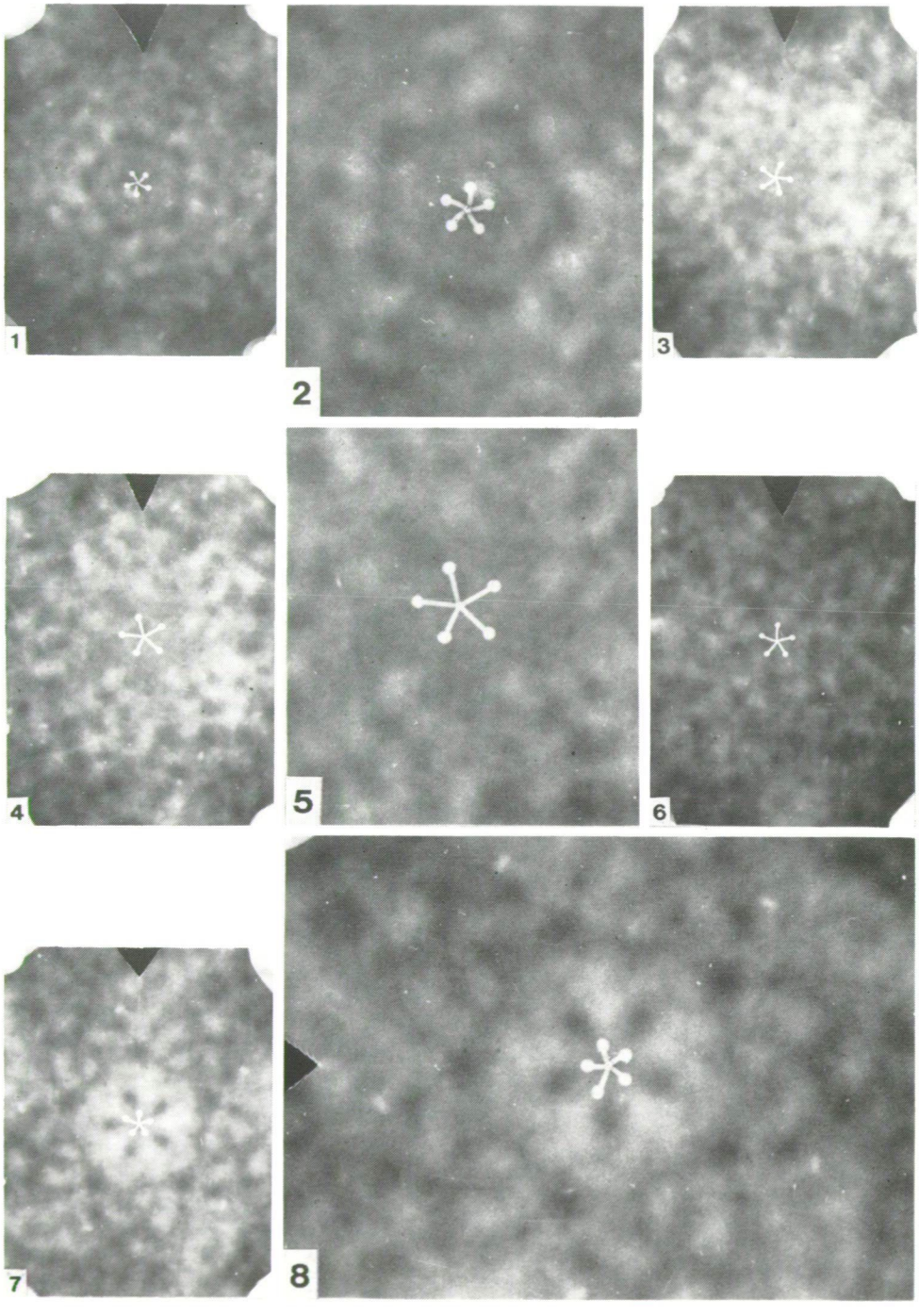
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◀ Plate 5.3.

Alnus glutinosa (L.) GAERTN.
The basic biopolymer unit after rotation.
1., 3. Rotation: C.S.X.1/1—10.5.5., 500.000 x.
2. Rotation: C.S.X.1/1—10.5.5., x 1 Million.
4., 6. Rotation: C.S.Z.2/10.5.5., 500.000 x.
5. Rotation: C.S.Z.2/10.5.5., x 1 Million.
7. Rotation: C.S.X.3/1—20.5.5., 500.000 x.
8. Rotation: C.S.X.3/1—20.5.5., x 1 Million.

fixing the photograph paper also serves valuable information. In figs. 6, 7, the fixation of the centre was not strong enough during the rotation. This "defective" attempt seems to serve not also neglectable information as it is well illustrated in Plate 5.2., fig. 7. In this case the connecting PENROSE-like units may be recognized. It is interesting that the results of a completely different, not really well rotation are essentially similar — it is illustrated in Plate 5.2., fig. 8.

In consequence another important methodical question emerged: that the secondary points are outside the AP axis. This probably results from the position of the pentagonal polygon biopolymer unit.

C.P.5.B.5.5 rotation seemed to be not necessary in this case.

C.P.5.A.5.10. (Plate 5.2., figs. 2, 4, 5)
This was also repeated twice. In detail some differences are illustrated in figs. 4. and 5.

2. RESULTS OF THE SECONDARY ROTATIONS

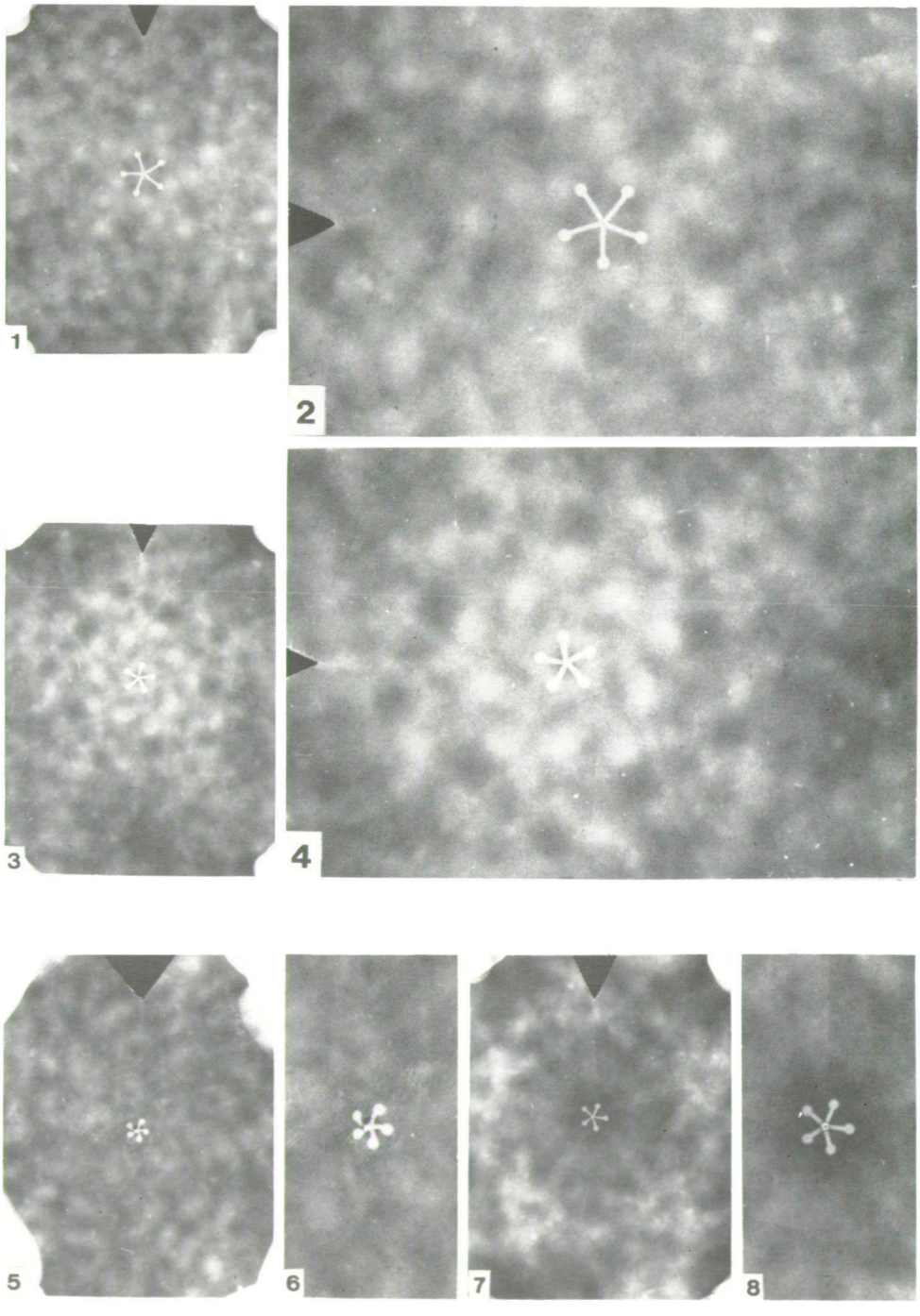
Taking into consideration the previous results, all kinds of rotations were repeated twice.

As new methodical establishments the following can be pointed out:

In the case when the PA = PX axis doesn't cross the secondary point, "theoretical" rotation points can be marked out. This is the crossing point of the circle line of the secondary symmetry points and the PX axis. This point is indicated by the first and last biopolymer of the circle, e.g.: 3/1—20 indicates the point between the first and the 20th secondary points of the third circle.

When it is impossible to count the points of symmetries (the circle is not complete) then the point is indicated with "n" after the number of the circle. In this cases this secondary point is the centre of this kind of rotations. We have to emphasize that in such a case a scheme is obligatory.

C.S.X.1/1—10.5.5. (Plate 5.3., figs. 1—3, text-fig. 5.1.)
Minor mistakes are causally made during the rotations. The shadows of the pin well indicate this phenomenon. Regarding the details there are differences but characteristic larger pentagons have appeared. Particularly picture 3 represents well the points of symmetry. The axis intersects the line connecting the two apexes of the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◀ Plate 5.4.

Alnus glutinosa (L.) GAERTN.
The basic biopolymer unit after rotation.
1. Rotation: C.S.5.Z.3/1.5.5., 500.000 x.
2. Rotation: C.S.5.Z.3/1.5.5., x 1 Million.
3. Rotation: C.S.5.Y.3/1.5.5., 500.000 x.
4. Rotation: C.S.5.Y.3/1.5.5., x 1 Million.
5—8. Rotation: C.S.5.Y.4/n.5.5.
5. 500.000 x.
6. x 1 Million.
7. 500.000 x.
8. x 1 Million.

large pentagon. It is worth mentioning that numerous secondary points can be investigated which are pro parte dark or light. Of course, these secondary points open new chance of further symmetry operations.

C.S.Z.2/10.5.5. (Plate 5.3., figs. 4—6, text-fig. 5.1.)

As regards the methodical situation this is the same as previously described. But at the case of both rotations illustrated in figs. 4 and 6, Plate 5.3., the appeared points of the PENROSE-like biopolymer arrangement can be recognized. This biopolymer organization is well illustrated in fig. 4., Plate 5.3. Further characteristic secondary points of symmetry have appeared.

C.S.X.3/1—20.5.5. (Plate 5.3., figs. 7, 8, text-fig. 5.1.)

In the direction of the left hand oriented peculiar pentagon was reinforced. Around this pentagon a large light pentagonal structure has appeared. Round the light structure numerous supplementary points of symmetry can be observed. The PENROSE-like arrangement is to be recognized, too.

For the point of symmetry 3/1 we have made two kinds of rotations: the "Y" and the "Z" as well.

C.S.5.Z.3/1.5.5. (Plate 5.4., figs. 1, 2, text-fig. 5.1.)

In the figs. 1, 2, of Plate 5.4. the two larger pentagons are well shown. The globular units of the light one are oriented at the rotation axis. The orientation of the dark pentagon is slightly in the left hand direction. There are several further new characteristic points of symmetry — light and dark as well.

C.S.5.Y.3/1.5.5. (Plate 5.4., figs. 3, 4, text-fig. 5.1.)

A remarkable difference can be established in contrast to the previous kind of rotations. Three characteristic pentagons have appeared, which are arranged concentrically. The inner one is composed of five dark points of symmetry. The following is light and ten points can be counted. Another large dark pentagon follows this. At the apices of this pentagon further units can be observed in pentagonal arrangement.

C.S.5.4/n.5.5. (Plate 5.4., figs. 5—8, text-fig. 5.1.)

These points of symmetry are the farthest ones from the basic "P" point. It is well-shown that the rotation illustrated in pictures 5,6 is not completely perfect. Peculiar and interesting pentagons have appeared; dark points surrounded by light circles. The approximatively perfect rotation has resulted interesting pentagons with straight sides.

General conclusions

1. The pentagonal polygons becoming regulated in the structure in the inner part of the plant cell wall result more points of symmetries contrast with the extremely disintegrated bordering part of the exine.
2. The new methodical supplements can be useful probably later. E.g.: After the C.P.5.A.5.10. rotation the secondary points are exclusively outside the PA (= AP) axis.
3. The repetitions of the same type of rotations seem to be extremely necessary in this case. It is worth mentioning that the not perfect rotation has resulted in interesting supplementary points of symmetries and configurations.

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